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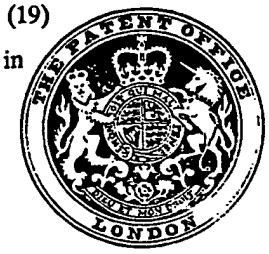

(72) Inventor: OLIVIER DE RHAM

(54) PROCESS FOR THE PREPARATION OF A VEGETABLE PROTEIN EXTRACT

(71) We, SOCIÉTÉ DES PRODUITS NESTLÉ S.A., a Swiss Body Corporate of Vevey, Switzerland, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:-

The present invention relates to processes for the preparation of vegetable protein extracts such as those which are known by the name of isolates (at least 85% by weight of proteins) or concentrates (65 to 85% by weight of proteins).

In order to obtain an extract rich in vegetable protein (e.g. an isolate or concentrate), the seeds or other elements of the vegetable are crushed to produce a flour; if the seeds have been treated beforehand with a solvent for fats, for example, n-hexane, a "defatted" flour is obtained.

The term "isolate" as used herein means a vegetable protein extract obtained by solubilisation of the protein component of a vegetable flour in an aqueous medium at a pH different from the isoelectric pH value of the protein, separation of insoluble material and adjustment of the pH to the isoelectric value to precipitate the protein. Thus, the flour, whether it has been defatted or not, may be contacted either with an alkaline medium (pH of 7 to 14) or with an acidic medium (pH of 1 to 3.5), the insoluble residue separated out and washed, and the washing water mixed with the soluble fraction. This soluble fraction may then be neutralised to the isoelectric pH value of the protein which under these conditions is precipitated in good yield and with good specificity. In the case of soya-bean, for example, contacted with an alkaline medium of pH 8 and precipitated at pH 4.5, 80 to 90% by weight of the dissolved protein is recovered in the isolate which then has a protein content of at least 85% by weight of dry matter. The rest of the dry matter comprises soluble carbohydrates, salts and organic compounds linked to the proteins. Of these phytic acid (myo-inositol-hexaphosphoric acid) represents 1.5 to 2% by weight of the dry matter.

The term "concentrate" as used herein means a vegetable protein extract prepared by washing a vegetable protein flour with an aqueous medium at the isoelectric pH of the protein and recovering the washed solid residue. Thus, for example, the defatted flour may be washed with an aqueous medium such as water at the isoelectric pH value of the protein and the solid residue rich in protein separated from the supernatant. The dry matter of this residue comprises polysaccharides, as well as salts and organic compounds, 1.2 to 1.7% by weight of which is phytic acid.

In both cases, the protein fraction contains active biological proteins, including, *inter alia* a considerable quantity of a trypsin inhibitor.

The presence of phytic acid and the trypsin inhibitor, has a deleterious effect on the value of the protein extract as a nutrient. In fact, the trypsin inhibitor can be the cause of hypertrophy of the pancreas and it can also be responsible for a reduction in the growth rate of young rats.

Phytic acid is capable, optionally in combination with proteins, of forming complexes with many metal cations essential to life, such as calcium, iron, magnesium and zinc, and therefore reduces their availability and resorption in the intestine.

Although protein extracts, having a low content of at least one of the above-mentioned undesirable substances, have been obtained, these extracts have only been prepared by the use of enzymatic or complex technological treatments,for example, ultrafiltration or by incubating the raw material for long periods at temperatures above ambient temperature. The process of the present invention provides an effective and simple method for obtaining

high quality vegetable protein extracts in contrast to prior art methods.

The present invention is based on the discovery that a vegetable protein extract having a low trypsin inhibitor content, a low phytic acid content and a reduced oligosaccharide content may be prepared by separation of that component of the vegetable flour, isolate or concentrate which is insoluble in a medium having a pH in a narrow range which is slightly greater than the isoelectric pH from components which are soluble in such a medium.

Thus according to one feature of the present invention there is provided a process for the preparation of a vegetable protein extract from a vegetable flour, isolate (as herein defined) or concentrate (as herein defined) which comprises separation of that component of the vegetable flour, isolate or concentrate, constituting the desired protein extract, which is insoluble in an aqueous medium having a pH of from the isoelectric pH (as herein defined) + 0.5 pH units to the isoelectric pH (as herein defined) + 1.2 pH units, from components which are soluble in said medium.

"Isoelectric pH" as used herein means the isoelectric pH value of the protein in question or where a mixture of proteins is concerned, the average isoelectric pH value, as identified by the solubility minimum. This pH value is about 4.5 for the majority of vegetable proteins for example, soya-bean and cotton, which have not undergone any special treatment other than rendering soluble (i.e. raw vegetable proteins). The pH value range in such cases thus extends from 5.0 to 5.7, preferably from pH 5.3 to 5.5.

In one embodiment of the present invention the process is conveniently effected such that a vegetable protein extract is obtained by washing the flour, isolate or concentrate with the said aqueous medium and recovering the residual insoluble component as the extract.

In a further embodiment of the present invention i) the vegetable flour is suspended in an alkaline aqueous medium having a pH of from 7 to 14 or in an acidic aqueous medium having a pH of from 1 to 3.5; and ii) the fraction of the flour which is soluble in said medium is isolated and the said vegetable protein extract is separated therefrom by precipitation at a pH of from the isoelectric pH + 0.5 pH units to the isoelectric pH + 1.2 pH units.

Thus, the process according to the present invention differs from conventional processes in that the working pH value is not the isoelectric pH value, but a substantially higher pH value. Although use of this higher pH value does entail a very slight reduction in the quantitative yield (i.e. the percentage quantity of protein recovered) this is more than compensated by the improvement in the quality of the proteins recovered as described above.

It has been established that in general the quantitative yield may be reduced by 10 to 20% by weight, but that the phytic acid and trypsin inhibitor content are reduced by a factor of from 2 to 4, depending on the pH value used. The oligosaccharide content is also reduced.

As is known, the vegetable substances from which valuable protein extracts may be obtained are plants especially leguminous and oleaginous plants such as, for example, soya-bean, sunflower, maize, horse bean, Lima bean, castor bean, cotton, croton, lupin, sesame, peanut, cow pea (*Vigna sinensis*) and chick-pea. The flours, preferably defatted, used as starting product, have been described above.

The concentrates or isolates for use as starting products and preferably further treated by washing have also been defined above. These latter starting products have all been obtained by the traditional method at the isoelectric pH value.

The pH value at which the vegetable protein extract is obtained is from the isoelectric pH + 0.5 pH units to the isoelectric pH + 1.2 pH units, preferably from the isoelectric pH + 0.8 pH units to the isoelectric pH + 1.0 pH units and may be adjusted by any convenient reagent, for example, hydrochloric acid, phosphoric acid, an acetate buffer, sodium hydroxide solution, potassium hydroxide solution or a carbonate buffer, as appropriate. The pH value is the determining factor in enabling the protein extract desired to be obtained in both quantity and quality. With regard to the use of soya-bean, for example, the phytic acid and trypsin inhibitor content below pH 5.0 is scarcely reduced at all, but above pH 5.7 the quantitative losses become too large and the trypsin inhibitor content again becomes too high.

The processes according to the present invention does not require any special apparatus or any costly reagents or treatments or any modification of existing production lines.

Separation of soluble and insoluble fractions may be carried out conventionally, for example, by filtration decanting, centrifuging or any other convenient technique. The insoluble fraction obtained may, if desired, be washed, in water or, advantageously, with an aqueous solution having a pH value from the isoelectric pH of the original flour, isolate or concentrate (as herein defined) + 0.5 pH units to the isoelectric pH of the original flour, isolate or concentrate (as herein defined) + 1.2 pH units.

The protein extracts can be used as such or after neutralisation, optionally after standardisation of their composition. They can also be dried (e.g. by spray-drying or lyophilisation). They can be used in the food industry, especially in human foods, notably in dietetics. Their nutrient value is considerably improved. Thus, according to a further feature of the present invention there is provided a food composition comprising *inter alia* a vegetable protein

extract prepared according to the process of the invention.

The following examples illustrate the processes according to the invention. In these examples the percentages are expressed in percentages by weight and the values according to the invention are identified by an asterisk in the comparison tables.

Example 1

Soya-bean concentrates

Concentrates are prepared by suspending a flour from dehulled and defatted soya-beans in water in the proportion of 100 g of flour in 1900 g of distilled water. This suspension is stirred at ambient temperature for 1 hour and 6 equal fractions, each of 300 g, are then withdrawn, the pH values of which are adjusted by means of hydrochloric acid to the pH values 4.5, 5.0, 5.3, 5.5, 5.7 and 6.0. These fractions are then centrifuged at 3000 G for 15 minutes and the centrifuged deposit is dried by lyophilisation. The quantity and composition of these dried fractions are given in the following table.

Quantity of starting product: 15 g per test		*	*	*	*	
- pH value reached of acidification	4.5	5.0	5.3	5.5	5.7	6.0
- quantity of dried product	10.4g	10.0g	9.7g	9.2g	8.5g	7.3g
- quantity of nitrogen	1.22g	1.18g	1.16g	1.10g	1.00g	0.57g
- % protein (by weight)	73.0%	73.7%	74.3%	74.3%	73.7%	48.8%
- % phytic acid (by weight)	1.49%	0.97%	0.75%	0.67%	0.64%	0.50%
- trypsin inhibitor level (arbitrary units per mg of nitrogen)	370	295	225	185	190	244

It is clear that concentrates prepared at a pH value between 5.0 and 5.7 are better than those prepared at pH value 4.5, without the reduction of yield becoming unacceptable, as at pH value 6.0.

Example 2

Soya-bean isolates (by washing) and soya-bean concentrates

10 g of the following products are subjected to washing with 50-200 g water at pH value 5.5. The suspension thus obtained is centrifuged at 3000 G for 15 minutes; the insoluble residue is recovered and dried.

Starting products	Weight of insoluble product	% Protein (by weight)	% Phytic acid (by weight) obtained	% Phytic acid (by weight) at the start
Experimental isolate (pH 4.5)	8.95 g	96.9%	1.20%	1.84%
Commercial isolate (Promine [registered Trade Mark] R)	8.5 g	95.0%	1.13%	1.40%
Experimental concentrate (pH 4.5)	8.83 g	66.5%	0.89%	1.31%
Commercial concentrate (Danpro [registered Trade Mark] H)	8.6 g	75.0%	1.17%	1.78%

Example 3

Soya-bean isolates (by precipitation)

From a flour of dehulled and defatted soya-bean isolates are prepared as follows: 100 g of flour are suspended in 1900 g of water and the pH value is then adjusted to 8.0 by means of sodium hydroxide. This suspension is centrifuged for 15 minutes at 3000 G to obtain 1740 g of solution containing 90% by weight of the proteins of the flour. Six identical fractions, each of 280 g, are then prepared, the proteins of which are precipitated by the addition of hydrochloric acid up to the following pH values: 4.5, 5.0, 5.3, 5.5, 5.7 and 6.0. These fractions are then centrifuged at 3000 G for 15 minutes and the centrifuged deposits are recovered and dried by lyophilisation. The results are set out as in Example 1.

Quantity of starting product: 16 g per test

- pH value reached after acidification	4.5	5.0	5.3	5.5	5.7	6.0
- quantity of dried product	6.25g	6.15g	5.5g	5.25g	5.1g	1.55g
- quantity of nitrogen	0.92g	0.90g	0.82g	0.76g	0.72g	0.20g
- % protein (by weight)	92.0%	91.4%	93.2%	90.5%	88.3%	80.6%
- % phytic acid (by weight)	2.03%	1.14%	0.82%	0.68%	0.58%	0.76%
- % of trypsin inhibitor (arbitrary units per mg of nitrogen)	375	225	205	155	160	190

Example 4

Isolates of soya-bean, sesame, peanut and chick-pea (by precipitation)

a) a commercial defatted soya-bean flour (Baker's Nutrisoy from Archer Daniel Midlands), not treated beforehand for heat and thus possessing an elevated nitrogen solubility index, is suspended at pH 8.0 at the rate of 60g of flour to 540g of water. After elimination of the insoluble part by centrifuging half of the supernatant is acidified at pH 4.5 and the other half at pH 5.5.

b) the same process is applied to another commercial defatted soya-bean flour (Soyafluff 200 W from Central Soya). The results are qualitatively identical to those of Example 4a, but the fact that the said flour was treated for heat results in lower quantitative yields.

c) the process concerned is applied to a sesame flour. Lowering of the phytic acid rate is obvious despite the very low solubility of the raw material.

d) the process is also applied successfully to a defatted peanut flour. The quantity of protein recovered is, in this case, the same at both pH values.

e) the process is likewise realised by means of ground chick-peas; lowering of the phytic acid content is indisputable here, also.

Examples	Starting product	Protein precipitated at pH 4.5	Phytic acid content (by weight)	Protein precipitated at pH 5.5 *	Phytic acid content (by weight) *
4a	Bakers Nutrisoy	8.3 g	2.2%	6.4 g	0.77%
4b	Soyafluff 200W	6.4 g	2.2%	4.5 g	0.88%
4c	Sesame	1.12 g	2.9%	1.05 g	0.86%
4d	Peanut	13.4 g	1.6%	13.6 g	0.70%
4e	Chick-peas	3.5 g	1.5%	3.0 g	0.44%

Example 5

Soya-bean isolates (by precipitation)

5 kg of defatted soya-bean flour are suspended in 95 kg of water and the pH value is then adjusted to a value of 8.2 and the insoluble fraction is separated by centrifuging in a machine from the firm Alfa-Laval. The juice collected which contains 85% by weight of the total nitrogenous material of the soya-bean is then acidified at pH 5.5, mixed to facilitate precipitation and centrifuged in the same machine as above. The residue is recovered and dried by lyophilisation. 1.55 kg of isolate are thus obtained with 92.9% by weight of protein, that is, 66% by weight of the total nitrogenous material of the soya-bean. This isolate contains 0.61% by weight of phytic acid and 96 units of trypsin inhibitor (measured according to Kakade and coll., Cereal Chem. *51*, 376 (1974)) per mg of nitrogen.

Its PER (protein efficiency ratio in relation to a casein standard of PER = 3.2) is 2.18, a value which changes to 2.66 after thermal treatment for 10 minutes at 100°C.

By way of comparison, an isolate prepared under identical conditions by acidification at pH 4.5 gives 2.26 kg of protein, that is, 78% by weight of the nitrogenous material of the soya-bean. It contains 1.8% by weight of phytic acid and 350 units of trypsin inhibitor (according to Kakade and coll.) per mg of nitrogen. Its PER is only 1.66 and after thermal treatment for 10 minutes at 100°C it does not exceed 2.11.

A commercial isolate, "Promine" R, has a PER of 1.1 and 1.7 after heating under the conditions set out above.

Example 6
Soya-bean and peanut isolates (by precipitation)

a) The preparation described in Example 4a is repeated, with the difference that the defatted soya-bean flour (60 g) is suspended in an aqueous medium of sodium hydroxide given a final pH value of 12.0. After elimination of the insoluble part by centrifuging half the supernatant is acidified at pH 4.5 and the other half at pH 5.5.

b) The preparation of a peanut isolate, as described in Example 4d, is repeated under the pH value conditions described above, namely, 11.5. After elimination of the insoluble part by centrifuging half the supernatant is acidified at pH 4.5 and the other half at pH 5.5.

Examples	Starting product	Protein precipitated at pH 4.5	Phytic acid content (by weight)	Protein precipitated at pH 5.5 *	Phytic acid content (by weight) *
6a	degreased soya-bean flour	11.0 g	0.53%	11.1 g	0.10%
6b	degreased peanut flour	13.0 g	1.47%	12.4 g	0.47%

By way of comparison, the trypsin inhibitor content of the soya-bean isolate at pH 5.5 of Example 6a is 229 units against 375 units for the isolate prepared under the same conditions, but precipitated at pH 4.5.

Example 7
Soya-bean and peanut isolates (by precipitation)

a) The preparation described in Example 4a is repeated, with the difference that the defatted soya-bean flour is suspended in an aqueous medium of hydrochloric acid to obtain a pH value of 2.5. After elimination of the insoluble parts by centrifuging half the supernatant is acidified at pH 4.5 and the other half at pH 5.5.

b) Preparation of a peanut isolate described in Example 4d is repeated under the pH value conditions described above, namely, 2.5. After elimination of the insoluble part by centrifuging half the supernatant is acidified at pH 4.5 and the other half at pH 5.5.

Examples	Starting product	Protein precipitated at pH 4.5	Phytic acid content (by weight)	Protein precipitated at pH 5.5 *	Phytic acid content (by weight) *
7a	degreased soya-bean flour	8.9 g	1.73%	8.2 g	0.69%
7b	degreased peanut flour	7.3 g	1.85%	7.3 g	0.77%

By way of comparison, the trypsin inhibitor content is 242 units for the soya-bean isolate of Example 7a, when precipitated at pH 5.5, against 443 units when precipitated at pH 4.5.

Example 8
Soya-bean isolate (by precipitation)

The mode of execution of Example 6a is repeated at a larger scale from 10 kg of defatted soya-bean flour suspended in an aqueous medium of sodium hydroxide giving a final pH value of 11.5. After elimination of the insoluble product there are obtained by precipitation at pH 5.5, 4.0 kg of soya-bean isolate containing 3.6 kg of proteins and having a phytic acid content of 0.18% by weight.

Example 9
Whole soya-bean isolate (by precipitation)

Suspended in 190 g of water are 10 g of dehulled and ground whole soya-bean seeds, after which the pH value of the suspension is adjusted to 8.0. The insoluble substances are then eliminated by centrifuging at 2000 g for 10 minutes. The fatty substances separated on the surface are added to the solution obtained after centrifuging, the pH value of the said solution being adjusted to 5.5.

The precipitate obtained (4 g after drying) is an isolate which contains only 0.32% by weight of phytic acid for a protein content of 62% by weight of total dry substances, that is, approximately 83% by weight of non-fatty dry substances.

By way of comparison, there are obtained by precipitation at pH 4.5, 4.9 g of isolate containing 57% by weight of proteins in total dry substances and 1.32% by weight of phytic acid.

Example 10
Whole soya-bean concentrate

A whole soya-bean concentrate is prepared by suspending 10 g of dehulled and ground whole soya-bean seeds in 190 g of water and then adjusting the pH value to 5.5. The insoluble fraction is then separated by centrifuging at 2000 g for 10 minutes, the fatty substance of the supernatant being added to the centrifuged deposit. There is thus obtained after drying a whole soya-bean concentrate (6 g) which contains 51% by weight of proteins in total dry substances, that is, 68% by weight in non-fatty dry substances, and 0.31% by weight of phytic acid.

WHAT WE CLAIM IS:

1. A process for the preparation of a vegetable protein extract from a vegetable flour, isolate (as herein defined) or concentrate (as herein defined) which comprises separation of that component of the vegetable flour, isolate or concentrate, constituting the desired vegetable protein extract, which is insoluble in an aqueous medium having a pH of from the isoelectric pH (as herein defined) + 0.5 pH units to the isoelectric pH (as herein defined) + 1.2 pH units from components which are soluble in said medium.

2. A process as claimed in claim 1 wherein the vegetable protein extract is obtained by washing the flour, isolate or concentrate, with the said aqueous medium and recovering the residual insoluble component as the extract.

3. A process as claimed in claim 1 wherein i) the vegetable flour is suspended in an alkaline aqueous medium having a pH of from 7 to 14 or in an acidic aqueous medium having a pH from 1 to 3.5; and ii) the fraction of the flour which is soluble in said medium is isolated and the said vegetable protein extract is separated therefrom by precipitation at a pH of from the isoelectric pH + 0.5 pH units to the isoelectric pH + 1.2 pH units.

4. A process as claimed in any one of the preceding claims wherein the said precipitation is effected at a pH in the range from the isoelectric pH + 0.8 pH units to the isoelectric pH + 1.0 pH units.

5. A process as claimed in any one of the preceding claims wherein the pH of the aqueous medium is adjusted by the use of hydrochloric acid, phosphoric acid, an acetate buffer, a carbonate buffer, a sodium hydroxide solution or a potassium hydroxide solution.

6. A process as claimed in any one of the preceding claims wherein the flour, isolate or concentrate is a leguminous or oleaginous vegetable flour, isolate or concentrate.

7. A process as claimed in any one of claims 1 to 5 wherein the flour, isolate or concentrate is a flour, isolate or concentrate of soya bean, sunflower, maize, horse bean, lima bean, castor bean, cotton, croton, lupin, sesame, peanut, cow pea or chick pea.

8. A process as claimed in any one of the preceding claims wherein the vegetable protein extract obtained is washed with water or with an aqueous solution having a pH of from the isoelectric pH of the flour, isolate or concentrate (as herein defined) + 0.5 pH units to the isoelectric pH of the flour, isolate or concentrate (as herein defined) + 1.2 pH units.

9. A process as claimed in any one of the preceding claims wherein the vegetable protein extract obtained is dried.

10. A process as claimed in any one of the preceding claims substantially as herein described.

11. A process for the preparation of a vegetable protein extract as claimed in claim 1 substantially as herein described in any one of the Examples.

12. A vegetable protein extract when prepared by a process as claimed in any one of the preceding claims.

13. A food composition comprising *inter alia* a vegetable protein extract as defined in claim 12.

For the Applicants
FRANK B. DEHN & CO.
Chartered Patents Agents
Imperial House
15-19 Kingsway
London WC2B 6UZ

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